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EFFECTS OF REMOVING THE GERM-CELL DETERMINANTS FROM THE EGGS OF SOME CHRYSOMELID BEETLES. PRELIMINARY REPORT.<sup>1</sup>

R. W. HEGNER.

A number of experiments were made during the spring and summer of 1908 in order to test the conclusions reached in a previous study of the origin and early history of the germ-cells in *Calligrapha multipunctata* and several other chrysomelid beetles. These experiments suggested some interesting hypotheses which will be verified or disproved as soon as more material can be obtained. The work was begun at the Zoölogical Laboratory of the University of Wisconsin and continued at the Marine Biological Laboratory, Woods Hole, Mass.,<sup>2</sup> and at the Zoölogical Laboratory of the University of Michigan.

In a paper soon to be published<sup>3</sup> I have shown that there is an intimate connection between the primordial germ-cells and a disc of granules lying near the posterior end of the eggs of *Calligrapha multipunctata*, *C. bigsbyana*, *C. lunata* and *Leptinotarsa decemlineata*. This study led to an attempt to secure confirmatory experimental evidence. A brief account of the structure of the beetle's egg and the early history of the germ-cells is necessary for a clear understanding of the method of procedure and of the results.

The freshly laid eggs of these beetles consist of a thin peripheral layer of cytoplasm (the "Kleinhautblastem" of Weismann) and a relatively large central mass composed of yolk globules with a small amount of cytoplasm filling the inter-

<sup>1</sup> Contributions from the Zoölogical Laboratory of the University of Michigan, No. 120.

<sup>2</sup> The writer is indebted to the Wistar Institute of Anatomy and Biology for the use of a room at the Marine Biological Laboratory, Woods Hole, Mass., during the summer of 1908.

<sup>3</sup> "The Origin and Early History of the Germ-Cells in some Chrysomelid Beetles." Accepted for publication by the *Journal of Morphology*.

deutoplasmic spaces. When the egg is deposited, the germinal vesicle, has already moved to a point near the ventral surface just a trifle anterior to the center of the egg; here polar-body formation takes place. One or more male nuclei are present near the anterior end of the egg. Suspended in the "Keimhautblastem" near the posterior end of the egg is a mass of deeply staining granules which I have called the "pole-disc." These granules occupy the inner portion of the peripheral cytoplasmic layer and cover about one eighth of the area at this end of the egg (Fig. 1). Surrounding the egg are the vitelline membrane and the chorion.

After the conjugation of the sperm-nucleus with the egg-nucleus, which occurs a few hours after the eggs are laid, the



FIG. 1.

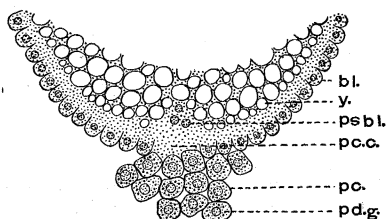


FIG. 2.

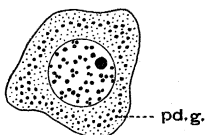


FIG. 3.

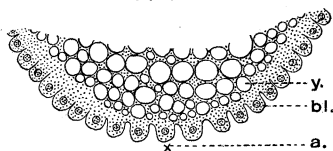


FIG. 4.

FIG. 1. A portion of the posterior end of an egg of *C. bigsbyana* in longitudinal section showing the arrangement of the pole-disc granules (*pd.g*). *y* = yolk; *khbl* = "Keimhautblastem."

FIG. 2. Longitudinal section through the posterior end of an egg of *C. bigsbyana* showing the primordial germ-cells (*pc*) containing pole-disc granules (*pd.g*). Nuclei which are prevented from forming blastoderm cells produce a pseudoblastodermic syncytium (*psbl*). The letters *pc.c* indicate the canal through which the pole-cells later migrate into the embryo. *bl* = blastoderm. *y* = yolk.

FIG. 3. A single primordial germ-cell showing pole-disc granules (*pd.g*).

FIG. 4. Longitudinal section through the posterior end of the egg of *C. lunata* described in Exp. 2a. The letter *a* indicates where germ-cells would be found in an uninjured egg. *bl* = blastoderm. *y* = yolk.

cleavage nuclei increase rapidly in numbers and migrate in all directions toward the periphery reaching the "Kleimhautblastem" at the expiration of eighteen hours. Those nuclei (sixteen in

number) that come in contact with the granules of the pole-disc, do not remain in the "Kleimhautblastem" and later become the centers of blastoderm cells as do the other nuclei but become completely surrounded by a thin layer of these darkly staining bodies and continue their migration until they are entirely separated from the egg. They then lie between the vitelline membrane and the blastoderm in a closely packed group (Fig. 2). Each of these sixteen cells ("pole-cells") has the appearance of the one shown in Fig. 3. They soon divide by mitosis to form thirty-two. During this division the pole-disc granules are equally distributed among the daughter cells. Another division increases the number to sixty-four. This number is reached at the end of twenty-four hours and no further divisions take place until about the time of hatching. The blastoderm cells increase rapidly in number and can be distinguished from the cells just described not only because of the absence of granules but also by the smaller size of their nuclei. No difficulty was experienced in tracing the sixty-four cells containing pole-disc granules until they became the definite germ-gland.

These phenomena give reasonable grounds for the following conclusions. All the cleavage nuclei in the eggs of the above named beetles are potentially alike until in their migration toward the periphery they reach the "Keimhautblastem." Then those which chance to encounter the granules of the pole-disc are differentiated by their environment, *i. e.*, the granules, into germ-cells; all the other cleavage products become somatic cells. The granules of the pole-disc are therefore either the germ-cell determinants or the visible sign of the germ-cell determinants. The term determinant is not used here in the Weismannian sense but simply is meant to describe the material which fixes the character of the cells.

At first an attempt was made to tie off the posterior end of freshly laid eggs by means of fine silk thread in order to remove the pole-disc. This proved impossible for in every case the pressure burst the chorion. It was later found that if the chorion is punctured with a needle at any point a small drop of the contents is forced out by the turgidity of the egg and can be removed with filter paper. The eggs of these insects are perfectly oriented

when laid. They were carefully placed with the posterior end up in small cavities in a block of paraffine. With the aid of a binocular microscope the point of a fine needle was then inserted at the center of this end, thus allowing that portion of the egg containing the pole-disc granules or primordial germ-cells to flow out. After removing this the eggs were placed in watch glasses and allowed to develop to the desired stage. Some difficulty was experienced in removing the proper amount of material and a number of the eggs either developed into shapeless masses of tissue or did not fix properly. These sources of error are in large part responsible for the failure to obtain more definite results.

#### I. THE EFFECTS OF REMOVING THE POLE-DISC GRANULES.

1. *C. multipunctata*, Exp. C.m. 1. — One egg of *C. multipunctata* was laid on April 15, 1908, and operated on while fresh. The portion removed from the posterior end was fixed on a slide and stained. It was found to contain the pole-disc granules. This egg did not hatch in the average period ( $5\frac{2}{3}$  days) and so was preserved at the age of 8 days.<sup>1</sup> The embryo appeared to be normal in every way, but on sectioning it was found to be lacking not only in germ-cells but also in other abdominal tissues. The mid- and hind-intestines were not completely formed and other neighboring parts were likewise deficient. It is evident that the germ-cell determinants were removed by the operation and that so much of the other organ-forming substances was also removed that the embryo failed to develop the corresponding tissues.

2. *C. lunata*, Exp. C. 1. — An egg of *C. lunata* which was laid at 12 o'clock noon July 16, was operated at 2 p. m. on the same day and fixed forty-nine hours later. It was stained in toto with hæmalum, drawn and then sectioned. Externally it resembled exactly embryos of this age developed from entire eggs. Germ-cells were found in the last two abdominal segments in a position similar to that occupied by them in normal embryos. However, there were not sixty-four, as was found to be the case in embryos of *C. multipunctata*, but only thirty germ-cells could be

<sup>1</sup> It has often been observed that when eggs of this beetle do not hatch the embryos live for several days after the expiration of the regular hatching period.

found. It seems probable that not all of the pole-disc granules were removed in the operation and that those which were left behind determined the character of the cells which encountered them, the result being a smaller number of germ-cells.

3. *C. lunata*, Exp. C. 3. — This larva hatched at noon on July 22, exactly six days after the egg was laid. This constitutes a period eight hours longer than has been computed as the average time for eggs of this species.<sup>1</sup> Apparently very little if any delay in development was caused by the operation. This larva was normal in external appearance and was as active as those hatched from eggs which had not been injured. It was killed when three days old. No germ-glands could be discovered on sectioning, but as several sections were ruined by an accident no incontestable statement as to their presence or absence can be made.

4. *C. lunata*, Exp. 2a. — This egg was laid and operated on at 10 a. m. July 24 and was fixed at the expiration of twenty-two hours. At this age a normal egg has a group of primordial germ-cells between the vitelline membrane and the blastoderm at the posterior end (Fig. 2). No germ-cells are present in the operated egg; the blastoderm cells at the posterior pole (Fig. 4) are less numerous than at other points on the surface indicating a delayed development in this region due to the removal of the "Keimhautblastem."

5. *C. lunata*, Exp. 2b. — This egg was laid and operated on at the same time as the egg in Exp. 2a. It was preserved when thirty-six hours old. At this stage in uninjured eggs the germ-cells are present as a group near the posterior end of the ventral groove. No germ-cells were found in the operated egg but a mass of yolk and cytoplasm was discovered outside of the vitelline membrane near the posterior end.

## II. THE EFFECTS OF REMOVING THE PRIMORDIAL GERM-CELLS.

In two experiments on stages shown in Fig. 2 eggs were punctured and the germ-cells allowed to flow out. The results indicate that all of the germ-cells were not removed in every case.

<sup>1</sup> For an account of the life histories of these beetles see R. W. Hegner, "Observations on the Breeding Habits of three Chrysomelid Beetles, *Calligrapha bigsbyana*, *C. multipunctata* and *C. lunata*," *Psyche*, Vol. 15, pp. 21-24.

It is the intention of the writer to repeat these operations next spring.

1. *C. lunata*, Exp. 6*b*. — An egg which was laid at 11 a. m. on July 23, was allowed to develop for twenty-five hours and then was operated on. It was preserved forty-four hours after the operation. Twenty-three germ-cells instead of sixty-four were found occupying the normal position in the embryo. Four of these twenty-three cells were in the posterior amniotic cavity while nineteen were scattered among the mesoderm cells in the last two abdominal segments. This difference in number can be accounted for by supposing that part of them were removed in the operation.

2. *C. lunata*, Exp. 6*c*. — A larva hatched from an egg which was laid and operated on at the same time as the egg in Exp. 6*a*. The larva was preserved at once. No germ-glands could be found in the sections. Several sections broke on the knife but these were from a part of the larva outside of the germ-gland region.

3. *L. decemlineata*, Exps. p. 1-7. — It is evident from the results of these experiments that either all of the primordial germ-cells were not removed from the egg during the operation or else some other cells have taken on the function of germ-cells. In one case (*L. decemlineata*, Exp. p. 2) it is certain that all the pole-cells were not removed. In two instances (Exps. p. 4 and p. 6) germ-glands were undoubtedly present. The method of operating therefore needs to be improved before decisive results can be secured.

### III. THE EFFECTS OF REMOVING OTHER PORTIONS OF THE EGG.

Only one experiment was made to note the effect of removing any part of the egg other than the posterior end. The anterior end of a freshly laid egg of *C. bigsbyana* (Exp. *C. b.* 1) was punctured and a large drop of the contents removed. A normal larva hatched from this egg, passed through the larval and pupal stages and is now hibernating as an adult.

The above experiments prove that a part of an insect's egg may be removed without preventing the development of the embryo and subsequent hatching of the larva. More work must be

TABLE GIVING THE NATURE AND RESULTS OF ALL THE EXPERIMENTS.

Name.	No. of Experiment.	Age of Egg When Operated.	Operation.	Interval Between Operation and Fixation.	External Condition of Embryo or Larva.	Sections.	Germ-Cells.	Histological Condition.
<i>C. multipunctata</i>	C. m. 1	fresh	pole-disc removed	8 days	normal	perfect	not present	imperfect
<i>C. lunata</i>	C. 1	2 hours	"	49 hours	"	"	fewer than normal	perfect
"	C. 2	"	"	97 "	"	"	"	"
"	C. 3	"	"	{ 9 days larva 3 days	"	several lost	"	"
"	2. a	fresh	"	22 hours	post. end altered	perfect	"	"
"	2. b	"	"	36 "	"	"	"	"
"	2. c	"	"	48 "	abnormal	"	"	"
"	2. d	"	"	72 "	ruined by accident	"	"	abnormal
"	2. e	"	"	7 days	abnormal	perfect	not found	abnormal
"	5. a	"	"	24 hours	ruined by accident	"	"	"
"	5. b	"	"	44 "	"	perfect	not found	abnormal
"	5. c	"	"	68 "	abnormal	"	"	"
"	5. d	"	"	9 days	"	"	"	normal
<i>L. decemlineata</i>	L. d. 1	"	"	{ 10 " 4 day larva	normal	"	"	"
"	L. d. 2	"	"	72 hours	"	several lost	"	"
"	L. d. 3	"	"	{ 6 days 1 day larva	"	"	"	"
"	L. d. j	"	"	48 hours	"	perfect	"	"
<i>C. lunata</i>	6. a	25 hours	primordial germ-cells removed	24 "	"	"	"	"
"	6. b	"	"	44 "	"	"	fewer than normal	"
"	6. c	"	"	{ 7 days 1 day larva	"	several lost	not found	"
<i>L. decemlineata</i>	p. 1	control	none	fixed at once	"	perfect	present	"
"	p. 2	26 hours	primordial germ-cells removed	16 hours	"	"	fewer than normal	"
"	p. 3	"	"	48 "	abnormal	"	not found	abnormal
"	p. 4	"	"	72 "	normal	"	present	normal
"	p. 5	"	"	{ 104 " 1 day larva	abnormal	several lost	not found	abnormal
"	p. 6	"	"	{ 8 days 4 day larva	normal	perfect	present	normal
"	p. 7	"	"	"	"	several lost	not found	"
<i>C. bigbyana</i>	C. b. 1	fresh	ant. end removed	"	hibernating (Oct. 30, 1908) as an adult	"	"	"



done before any final statement can be made as to the effects of removing the germ-cell determinants (pole-disc granules), but it seems probable that if all of these are taken from the egg no germ-cells will be produced. Previous researches have proven beyond a doubt that the pole-disc granules are taken up by the primordial germ-cells. It remains however for future experiments to decide whether these granules are really the germ-cell determinants or are non-essential in the differentiation of the cleavage products.

ZOÖLOGICAL LABORATORY,  
UNIVERSITY OF MICHIGAN,  
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